

Policy & Procedure (P&P)

Policy Title :

Antibody Titration

Department	Index No.	Scope
Laboratory & Blood Bank	LAB-070	All Blood Bank staff
Issue Date	Revision NO	Effective Date
1433/06/04	2	1440/07/23
Review Due Date	Related Standard NO.	Page Number#
1442/07/23	CBAHI (LB. 50.1.4)	4

01. Policy:

Titration is a semi quantitative method used to determine the concentration of antibody in serum. The usual application is in estimating antibody activity in all immunized pregnant women to determine whether and when to perform more complex invasive investigation of fetal condition.

02. Definition :

N/A

03. Purpose :

N/A

04. Procedure :

The titer of an antibody is obtained by testing serial dilutions of a serum against selected red cells. The result is expressed as the reciprocal of the highest serum dilution that causes macroscopic agglutination. Titration is most frequently performed as part of prenatal studies where the mother's serum is tested at intervals during pregnancy. A change in titer of more than two tubes is significant. A significant raise in titer in the first affected pregnancy suggests that the baby will very likely suffer from hemolytic disease of the newborn.

1. Specimen Requirements

Fresh serum obtained from a fully clotted specimen should be used. Plasma and hemolyzed specimens are unacceptable. Serum should be separated immediately from red blood cells. If testing is delayed, the specimen should be stored at 2-8°C and testing completed within 48 hours.

2. Materials

2. 1. Glass Test Tubes
2. 2. Automatic pipettes with disposable tips
2. 3. Water Bath
2. 4. Normal Saline
2. 5. Calibrated serologic centrifuge

3. Reagents

3. 1. Screening cells I, II, III
3. 2. Selected red cells carrying the appropriate antigen should be used. For example, the cells used in a titration of anti-D can be either screening cells I or II. The appropriate reagent cells may also be used for antibodies other than anti-D.
3. 3. 22% bovine albumin
3. 4. Anti-human serum.
3. 5. Coombs control cells "CCC".

4. Method

Incubation time, temperature and centrifugation conditions should be determined in preliminary evaluation of the antibody. Once determined these should be used consistently. Consult patient file for previous records.

Extremely careful pipetting technique is essential:

- 4.1. Label 12 test tubes according to serum dilution (1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, 1:512, 1:1024, 1:2048). A 1 in 1 dilution means one volume of serum undiluted; a 1 in 2 dilution means one volume of serum in a final volume of 2 or a 50% solution of serum in the diluents.
- 4.2. Deliver one volume of saline to all tubes except for first (1:1).
- 4.3. Deliver an equal volume patient serum to tubes 1 and 2 (dilutions 1:1 and 1:2)
- 4.4. Using a clean pipette tip mix the contents of tube 2 several times. Transfer one volume to the next tube (1:4 dilution).
- 4.5. Continue the mixing and transferring using the same technique through all dilutions and a clean pipette tip every time.
- 4.6. From the last tube remove one volume of the diluted serum and save for use in further dilutions, if needed.
- 4.7. Label another set of 12 tubes for the appropriate dilutions.
- 4.8. Using separate pipettes for each dilution transfer 2 drops of each diluted serum into the appropriately labeled tubes and 1 drop of the screening red cell carrying appropriate antigen.

- 4.9. Mix well and incubate at 37°C for 20 – 30 minutes after adding 2 drops of 22% bovine albumin into each tube.
 - 4.10. Spin the tubes according to the optimum spin time for the centrifuge. Observe for hemolysis. Gently suspend the cells and examine for agglutination. Record results.
 - 4.11. Wash the cells in the isotonic saline three times.
 - 4.12. Immediately add two drops of the anti-human serum and mix.
 - 4.13. Centrifuge according to the optimum spin time listed for the centrifuge.
 - 4.14. Resuspend the red cells by gentle agitation and examine macroscopically for agglutination.
 - 4.15. Control all negative tests by adding one drop of coombs control cells. Re-centrifuge and observe for macroscopic agglutination. These cells must agglutinate for a negative test result to be valid.
 - 4.16. Record results.
- 5. Quality Control**
- Reagent cells are to be tested with appropriate positive and negative controls on the day of testing and the results recorded.
- 6. Reporting Results**
6. 1. the highest dilution that produces macroscopic agglutination. The titer is reported as the reciprocal of the dilution level. A difference in titer between current and previous sample of at least two tubes is required to be considered a significant difference.
 6. 2. If there is agglutination in the tube containing the most dilute serum, the end point has not been reached and additional dilutions should be prepared and tested.
 6. 3. The prozone phenomenon may cause reactions to be weaker in the more concentrated serum preparations than in higher dilutions. To avoid misinterpretation of results, it may be preferable to examine first the tube containing the most dilute serum and proceed through the more concentrated samples to the undiluted specimens.
 6. 4. Measurements are more accurate with large volumes than with small volumes; a master dilution technique (mentioned in method) gives more reliable results than individual dilutions for a single set of tests.

05. Responsibilities :

- 05.1. All laboratory of Al-Qunfudah General Hospital.

06. Equipment & Forms

- 06.1. N/A

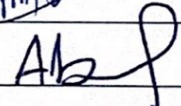
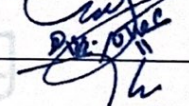
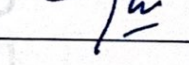
07. Attachment :

07.1. N/A

08. Reference

08.1. The Technical manual of the American Association of Blood Banks.

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